

## CLAIMS

1. A method for separating proteins comprising the steps of:

(a) adsorbing a target protein on an ion exchanger by allowing a  
5 sample containing the target protein to contact the ion exchanger under  
a first condition at high ion strength and at a pH outside of the  
vicinity of the isoelectric point of the target protein; and

(b) eluting the component adsorbed on the ion exchanger under a  
second condition at lower ion strength than in the first condition, and  
10 at a pH closer to the isoelectric point side of the protein in the  
first condition.

2. The method for separating proteins according to Claim 1  
comprising the step of using a buffer solution with a concentration of  
15 0.05 M or more in the first condition.

3. The method for separating proteins according to Claim 1  
comprising the steps of using a high concentration of the buffer  
solution comprising a combination of a weak acid and weak base in the  
20 first condition.

4. A method for separating proteins comprising the steps of:

(a) adsorbing a target protein on an ion exchanger by allowing a  
sample containing the target protein to contact the ion exchanger under  
25 a first condition at high ion strength and at a pH outside of the  
vicinity of an isoelectric point of the target protein; and

(b) eluting the component adsorbed on the ion exchanger under a  
second condition at ion strength equal to or lower than in the first  
condition, and at a pH closer to the isoelectric point side of the  
30 protein in the first condition.

5. The method for separating proteins according to Claim 1  
comprising the following step interposed between step (a) and step (b):

(c) washing the ion exchanger under a condition not eluting the  
35 target protein adsorbed on the ion exchanger.

6. The method for separating proteins according to Claim 5,  
wherein step (c) is applied under a substantially the same condition as  
in the first condition.

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7. The method for separating proteins according to Claim 1,  
wherein

the pH in the first condition is lower than the isoelectric point  
of the target protein,

10 the ion exchanger is a cation exchanger, and

the pH in the second condition is in the vicinity of or higher  
than the isoelectric point of the target protein.

8. The method for separating proteins according to Claim 1,  
15 wherein

the pH in the first condition is higher than the isoelectric  
point of the target protein,

the ion exchanger is an anion exchanger, and

20 the pH in the second condition is in the vicinity of or lower  
than the pH corresponding to the isoelectric point of the target  
protein.

9. The method for separating proteins according to Claim 1  
comprising the step of using a tris-succinate buffer in the first  
25 condition.

10. The method for separating proteins according to Claim 1,  
wherein the second condition comprises the step of using a buffer  
solution comprising a combination of the same acid and same base as in  
30 the buffer solution used in the first condition.

11. The method for separating proteins according to Claim 1,  
wherein the second condition comprises the step of using a buffer  
solution having a pH in the vicinity of the isoelectric point of the  
35 target protein.

12. The method for separating proteins according to Claim 1,  
wherein

the sample contains a plurality of target proteins, and  
5 step (b) comprises the step of continuously eluting the target  
proteins under a solvent condition corresponding to the isoelectric  
point of each protein.

13. The method for separating proteins according to Claim 1,  
10 wherein the protein is a glycoprotein.